Otolith Microchemistry as a Tool to Discriminate between River-Spawning Populations of Walleye (*Sander vitreus*) in Lake Erie

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Abstract

Effective management of Lake Erie's walleye (*Sander vitreus*) fisheries depends on an ability to understand the relative contributions of recruits from local spawning populations (stocks) to the fishable population. To achieve this goal, one must first find a means to discriminate between stocks so that the natal origins of older individuals (i.e., juveniles, adults) can be determined. Natural markers such as the microchemical composition of otoliths (calcified ear stones used for equilibrium and hearing) show great potential to differentiate between individuals originating in different spawning locations with unique water chemistries. However, this technique may be limited for river- or reef-spawning fishes whose young can quickly disperse from their natal site within a matter of days, before the natal-site signature is retained in the otoliths. To better understand the potential value of otolith microchemistry as a tool to discriminate between Lake Erie's two largest river-spawning stocks, we determined if young larval walleye retain in their otoliths the unique elemental signature that is characteristic of water in their natal (riverine) environment. Towards this end, we used Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) to analyze the otolith strontium concentration [Sr] of larvae collected from the Sandusky and Maumee Rivers during 1993-1995 and 2011-2012 (n=20 larvae/site/year), and daily ring counts in otoliths were used to age larvae. Our analyses show that otolith [Sr] differed between larvae produced in the Maumee versus Sandusky River, with [Sr] being significantly higher in the Sandusky. Although [Sr] increased with the fish age, differences between rivers were apparent even in otoliths from larvae as young as two days old. Given these findings, otolith microchemistry holds great potential to discriminate between Maumee and Sandusky River spawning stocks, even during years in which larvae are transported out of the river at a young age.

Introduction

Effective fisheries management can benefit immensely from an ability to determine which local spawning sub-populations, or stocks, are contributing recruits to the broader population. Such knowledge can help identify those stocks that are in need of protection, as well as those that could benefit from rehabilitation efforts. However, both freshwater and marine fishery management agencies face challenges in accomplishing this goal because a reliable method for discerning the natal origin of individuals in mixed aggregations, which are typical of older life stages, is lacking (Barnett-Johnson et al. 2008, Bradbury et al. 2008).

Artificial tagging offers one approach for discriminating among fish stocks and identifying natal origins. This approach, however, is less preferable than the use of "natural tags" (Campana 1999, Thorrold et al. 2002). Mark-recapture studies that involve the use of artificial tags are not feasible for species that are too small to be easily marked or that produce large numbers of offspring which experience high mortality during early life stages. Artificial tagging approaches also are less cost-effective than natural tagging approaches considering the effort required to tag and recapture sufficient numbers of individuals (Thorrold et al. 2002). Natural tagging approaches such as genetics and chemical composition of fish hard structures appear more advantageous, as all individuals are marked at birth and retain these tags for their lifetime (Campana 1999, Thorrold et al. 2002). Thus, the time and expense associated with manually tagging sufficient numbers of individuals is lessened, making the use of natural markers advantageous.

The microchemical composition of otoliths has been shown to be a valuable natural tagging approach in a variety fish populations, both freshwater (Wells et al. 2003, Brazner et al. 2004, Reichert et al. 2010) and marine (Secor et al. 1995, Dufour et al. 1998, Kennedy et al.

2002). Otoliths are calcified structures found within a membranous, fluid-filled capsule in the inner ear of teleost fishes that are used for balance and hearing. Otoliths grow continuously, forming daily growth rings in a concentric pattern that also can allow for reliable age determination (Campana and Neilson 1985, Campana 1999). In addition, otoliths can accumulate trace metals into their calcium-carbonate matrix in proportion to the concentration in the water (Elsdon and Gillanders 2003, Elsdon et al. 2008), and are acellular and metabolically inert (i.e., no reabsorption or alteration of the chemical composition occurs, if stored properly). In turn, these properties allow the microchemical composition of otoliths to serve as a reliable record of where an individual fish spent time prior to capture (Campana 1999, Campana and Thorrold 2001).

For otolith microchemistry to be useful as a stock discrimination tool, consistent water chemistry differences must exist between local spawning sites that are retained in the otolith. In addition, individual larvae must reside in these sites long enough to pick up the site's elemental signature in the otolith (Campana and Thorrold 2001). While much research in both freshwater and marine systems has demonstrated that water and otolith microchemical differences can be used to discriminate among stocks (Edmunds and Fletcher 1997, Kennedy et al. 2000), even for geographically close spawning stocks (Ludsin et al. 2006, Pangle et al. 2010), the question of how long larval fish need to spend in their natal site to allow their otolith microchemical composition to reflect that of the natal environment has yet to be fully answered. Clearly, if an individual disperses from its natal site prior to retaining an otolith microchemical signature that reflects its natal environment, the ability to identify its natal origin would be limited.

This issue seems most relevant to species (or stocks) that spawn in environments subject to high flows or water currents (e.g., rivers, unprotected reef environments) and have pelagic

early life stages (e.g., eggs, larvae), whose young also are small in size and/or demonstrate weak swimming abilities. Because previous studies have shown that it takes about 21 d for otolith elemental concentrations to reach equilibrium with the ambient water for juveniles and adults that have moved from one environment into another (Elsdon and Gillanders 2005, Lowe et al. 2009, Mion et al. 1998), the use of otolith microchemistry as a stock discrimination tool might be limited for river- or reef-spawning species whose young disperse from their natal site at an early age. However, the time required for otoliths to reflect the water's chemical signature has not yet been determined for larvae. Likewise, because female spawners can hydrate their eggs on the spawning grounds (Lowe et al. 2012), the time for the otolith to reflect water chemistry may be substantially less than the 21 d lag time associated with movement during older life stages.

Herein, we sought to determine whether otolith microchemistry can be used to discriminate between walleye (*Sander vitreus*) larvae captured from two river-spawning stocks (Maumee and Sandusky Rivers, OH) in Lake Erie's western basin. To do so, we quantified how otolith microchemistry varies with larval walleye age during multiple years over a two-decade period (1993-2012). We focused on strontium concentration [Sr] for this study because this element has been shown to differ between the Maumee (low [Sr]) and Sandusky (high [Sr]) Rivers in terms of water chemistry and the chemistry of yellow perch (*Perca flavescens*) otoliths (Ludsin et al. 2006, Pangle et al. 2010). Likewise, [Sr] has proven to be a reliable discriminator among stocks in other ecosystems, both freshwater and marine (Kennedy et al. 2000, Barnett-Johnson et al. 2008, Elsdon and Gillanders 2005). Because larvae produced in the Maumee and Sandusky Rivers reside in their natal river during the entire egg stage (Mion et al. 1998, Pritt et al. 2013), we hypothesized that otolith [Sr] differences would be apparent even in the youngest sampled larvae and that significant differences in [Sr] would be evident between spawning sites.

Materials and Methods

Study Species

Walleye (*Sander vitreus*) are of both economic and ecological importance in the North American Great Lakes. In Lake Erie, walleye support the largest recreational fishery, valued at over $800 million annually, as well as the second largest commercial fishery (Ohio Department of Natural Resources 2010). Walleye also is a dominant top predator in Lake Erie, showing an ability to regulate prey species composition via top-down control (Knight and Vondracek 1993). Because Lake Erie's walleye population and its recreational and commercial fisheries have been consistently weak since 2003, for reasons that are currently unknown (Vandergoot et al. 2010, Lake Erie Walleye Task Group 2014), Lake Erie fishery management agencies have been seeking to identify the factors responsible for recent poor recruitment, including the stocks that have been supporting the fishery of late (Cadrin and Silva 2005).

Lake Erie's walleye population is known to consist of multiple local spawning populations (i.e., stocks), including both river-producing and reef-producing ones (Mion et al. 1998, Roseman et al. 1996, Jones et al. 2003). The two focal stocks in this study, Maumee River and Sandusky River, are considered the two largest river-spawning stocks in the lake. Both rivers are subject to high flow rates when it rains within the watershed, which can transport larvae downstream at very young ages (Mion et al. 1998, M. DuFour, University of Toledo, personal communication). In turn, larval residence time in the natal river can vary from just a few days to several weeks, depending on flow rates (Mion et al. 1998).

Sample Collection and Measurement

To establish a relationship between water chemistry and otolith [Sr], we first analyzed [Sr] to calcium [Ca] ratio (Sr:Ca) data from water samples collected during 1993-1995 (National

Stream Water-Quality Monitoring Network; Alexander et al. 1996), 2001 (K. Hedges, B. Fryer, and S. Ludsin, unpublished data), and 2011-2012 (see Table 1). Samples were collected in the Maumee and Sandusky Rivers from < 1 m below the water surface (2-3 replicates weekly per year). Each 60 mL sample was filtered through a 0.45 μm filter and fixed with 2% nitric acid (HNO_3) before storage in acid-washed polypropylene bottles. Strontium concentration was subsequently determined using Inductively Coupled Plasma Optical Emission Spectrometry.

Larval walleye were collected in the Maumee and Sandusky Rivers during 1993-1995, 2001, and 2011-2012. Larvae (~20 per site per year) were collected weekly in the rivers throughout the larval production season (mid-March through early June) during all years. Larvae were collected with metered 1 x 2 m neuston nets (500 μm mesh), which were towed at the water surface (Mion et al. 1998). All larvae were preserved in 95% ethanol, which has been shown to have no effect on otolith Sr analysis (Hedges et al. 2004).

We measured the total length (TL; nearest 0.1 mm) of larvae before removing otoliths. Both sagittal otoliths were removed from each larva, with one otolith transferred onto a petrographic slide (for microchemical analysis; see below) and the other transferred to a microscope slide (for aging). To age fish, we counted daily growth rings after the hatch check (50-100x magnification, immersed in oil). We used the average of counts conducted by three different readers that had no prior knowledge of previous counts. Total length measurements and age determination were made using an image analysis system that consisted of NIS-Elements Microsoft Imaging software, a DS-Fi2 camera, and a Nikon Eclipse E200 compound microscope (Nikon Instruments Inc., Melville, NY).

Otolith Microchemical Determination

To quantify otolith [Sr], ~ 20 larval walleye from each river per year (Table 1) were processed. All otoliths from 1993-1995 and 2011-2012 were processed for microchemical analysis following the protocol of Ludsin et al. (2006), with the exception that we used a laser pulse instead of sonication as the final cleaning procedure on otoliths, as this laser pulse has been shown to be as effective in cleaning otoliths while at the same time reducing otolith loss (Gover et al. in press). The 2001 larvae followed the same processing protocol as Ludsin et al. (2006), as the otoliths were analyzed as part of a previous study (Hedges 2002). To prevent contamination, all otoliths were removed under a dissecting microscope in a ventilated Class 100 clean hood, with all materials that came in contact with otoliths (e.g., glass probes, petrographic slides) being washed in 13% nitric acid for 24 hours and rinsed in deionized water for 24 hours before use.

Strontium concentration in all otoliths was quantified using Laser-Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). With the exception of 2001 larvae, which were analyzed at the University of Windsor with a system consisting of a Continuum solid state Nd:YAG laser (see Hedges 2002), we used The Ohio State University's Trace Element Laboratory's LA-ICP-MS system to determine otolith [Sr]. This system consisted of a New Wave Research UP-193HE 193 ηm excimer laser with beam homogenizing optics and a ThermoFinnigan Element 2 Inductively Coupled Plasma Sector Field Mass Spectrometer. Each otolith was cleaned by a light laser pulse with 0% power setting and a properly sized (100 µm or 150 µm) laser beam to cover the whole otolith (Gover et al. in press) before quantifying [Sr]. To determine otolith [Sr], we ablated each otolith near the edge its outer edge to obtain the most recent [Sr] value, using three 15 µm laser spots (fluence of 13 J/cm^2, laser pulse energy of 23 µJ, and 40 laser pulses fired at a frequency of 10 Hz). Glass reference standards from the National

Institute of Standards and Technology (NIST 610 and 612) also were measured once every five otolith samples to standardize elemental concentrations.

Statistical Analyses

We were interested in quantifying differences in water Sr:Ca and otolith [Sr] between spawning sites through time, as well as well how [Sr] varied with larval walleye age within a site. Paired t-tests were used to evaluate whether mean Sr:Ca ratios were differed between the Maumee and Sandusky Rivers during 2001, 2011, and 2013; such analysis was not possible during 1993-1995 because water samples were not collected in replicate at either location. Analysis of variance (ANOVA) with a Tukey HSD multiple comparison procedure was used to determine whether [Sr] differed between spawning locations (main factors: location, year). We used linear regression analysis to quantify whether [Sr] predictably varied with larval walleye age during all years in which age data existed. In addition, we used analysis of covariance (ANCOVA) to determine whether the relationship between larval age and otolith [Sr] varied between spawning locations (response variable: otolith [Sr]; continuous predictor: age; covariate: location). The alpha-level for all analyses was set to 0.05.

Results

Water chemistry analyses showed that Sr:Ca differed between the Maumee and Sandusky Rivers during 2001, 2011, and 2012 (paired t-tests: all $p < 0.001$) with the Sandusky River always having significantly higher Sr:Ca levels than the Maumee River (Figure 1). While statistical tests could not be performed, owing to a lack of replication, Sr:Ca was visually higher in the Sandusky versus Maumee River during 1993-1995 (Figure 1). Annual variation in water Sr:Ca was observed with generally lower Sr:Ca values historically (1993-1995). 2011 in

particular had a relatively low Sr:Ca ratio in the Sandusky River compared to levels in other recent years (2001 and 2012).

The general water Sr:Ca trends observed in the Maumee and Sandusky Rivers were reflected in larval walleye otoliths (Figure 2). The [Sr] in otoliths was significantly higher in Sandusky River larvae than in Maumee River larvae across years (ANOVA with Tukey's HSD test: $p < 0.001$ for 1993-1995, 2001, and 2012; Figure 2), with exclusion of 2011 ($p = 0.82$). However, while no statistical difference was found between otolith [Sr] in the Sandusky River versus Maumee River in 2011, the mean was 1.5 times greater in the Sandusky River than the Maumee River (Figure 2).

We also found evidence to suggest that [Sr] increases with larval walleye age (Figure 3). Positive relationships (linear regression: all $p < 0.05$) between age and [Sr] were found in the Sandusky River during 1993-1995 and in both spawning locations during 2011-2012. By contrast, no relationship was found between age and [Sr] in the Maumee River during 1993-1995 ($p = 0.11$). Additionally, the y-intercept of the regression line for the Sandusky River was higher than for the Maumee River in both periods (ANCOVA, $p < 0.01$ in 1993-1995, $p = 0.28$ in 2011-2012), indicating that for larvae of any given age, otolith [Sr] was higher in the Sandusky River than the Maumee River.

Discussion and Conclusions

Our analyses showed that while [Sr] typically increased with increasing larval walleye age in both spawning locations, the y-intercept of this relationship was higher in the Sandusky River versus Maumee River during 1993-1995 and 2011-2012. In addition, we found that [Sr] in larval walleye otoliths differed between the Maumee and Sandusky Rivers, with significantly

higher levels being observed in the Sandusky River in nearly all years sampled across two decades (1993-1995, 2001, 2012; but not 2011). After discussing these results in more detail below, we comment on their implications for using otolith [Sr] as a stock discrimination tool in western Lake Erie and beyond.

Previous studies have shown that it takes ~21 d for the [Sr] in otoliths to come to equilibrium with the ambient water in juvenile and adult fish that move from one environment to another (Elsdon and Gillianders 2005, Lowe et al. 2009, Collingsworth et al. 2010). In our study, we also found that otoliths in larval walleye < 21 d of age had not yet reached equilibrium with the water, with exception of those individuals captured in the Maumee River during 1993-1995. The fact that Sandusky River walleye otoliths were not at equilibrium whereas some of the Maumee River ones were is logical given that previous research has shown that the time needed for the [Sr] in an otolith to come to equilibrium increases with increasing [Sr] in the water (Farrell and Campana 1996). We were, however, a bit surprised to find that even our youngest larvae (1-2 d of age, post-hatch) could be used to discriminate the Maumee River from the Sandusky River, given the long lag times associated with reaching equilibrium in juvenile and adult fish that have switched environments. We suspect that the ability for even young larvae to be useful in discriminating spawning sites is related to the fact that they resided in their natal river as eggs, with the eggs perhaps even being hydrated in the natal river. In this way, the accumulation of Sr in their otoliths for a period of several weeks as a pre-hatched individual (depending on water temperatures that influence development) may have be sufficient to allow for these individuals to characterize their natal site (Lowe et al. 2012).

Our analyses also showed that water Sr:Ca ratios and otolith [Sr] differed between the Maumee and Sandusky Rivers with the Sandusky River having higher values during most years.

These results support previous studies from both freshwater and marine ecosystems, which have shown [Sr] to be an effective stock marker (Dufour et al. 1998, Campana and Thorrold 2001, Kennedy et al. 2002, Ludsin et al. 2006, Lowe et al. 2009, Pangle et al. 2010). We did find, however, that water Sr:Ca ratios and corresponding otolith [Sr] varied among our study years, a finding also supported by the literature (Hamer et al. 2003, Pangle et al. 2010). Most prominently, we found that 2011 was a somewhat anomalous year, with otolith [Sr] not being significantly higher in Sandusky River relative to the Maumee River (although Sandusky River larvae did have a 1.5-fold higher [Sr] in their otoliths). The cause of this inconsistency is unclear. While it may due to sampling bias (i.e., only 7 larvae were processed in 2011 versus ~20 in other years), other factors also might be responsible. For example, high precipitation that led to increased river flows and river volumes in spring 2011 might have reduced water [Sr], and hence Sr available for uptake by walleye otoliths. Alternatively, growth rates during 2011 might have been anomalous relative to other years, which could have impacted the rate of Sr uptake (Kalish 1989, Collingsworth et al. 2010). Regardless of the cause, the variation that we observed in both water and otolith [Sr] points to the importance of quantifying [Sr] in otoliths on an annual basis (Elsdon et al. 2008, Gillanders 2002).

The implications of our findings are multiple. First, our study indicates that even very young larvae can be used to develop stock-specific markers. This knowledge can certainly benefit stock discrimination efforts both within and outside of the Lake Erie basin. Within Lake Erie, our results clearly show that larval walleye otolith [Sr] can be used to discriminate between the two largest river-spawning stocks during most years, a finding supported by similar research conducted with yellow perch (Ludsin et al. 2006, Collingsworth et al. 2010, Pangle et al. 2010). This ability to use very young larvae to discriminate spawning sites also may help in

discriminating open-lake reef spawning stocks whose larvae are subject to rapid dispersal at a young age by wind-driven water circulation (Zhao et al. 2009). Similarly, our findings also should have relevance to those estuarine and pelagic marine fishes that have pelagic larval stages, which might only reside in their natal river (bluefish *Pomatomus saltatrix,* Edmunds et al. 1999; Atlantic salmon *Salmo salar,* Kennedy et al. 2000; Southern garfish *Hyporhamphus melanochir,* Steer et al. 2010) or on their natal reef (French grunts *Haemulon flavolineatum,* Chittaro et al. 2004; butterflyfish *Chaetodon ulietensis* and surgeonfish Ac*anthurus triostegus,* Dufour et al. 1998) for a brief period. Second, our findings caution against making assumptions about the microchemical composition of larval otoliths, even when water chemistry differences are known. For example, despite significant differences in water chemistry between the Maumee and Sandusky River during 2011, otolith [Sr] differences were not statistically significant. While we plan to analyze additional 2011 samples to rule out sampling bias, high inter-annual variation in otolith elemental concentrations is not uncommon (Gillanders 2002, Pangle et al. 2010). Thus, until strong consistency in elemental signatures between spawning locations has been definitively established, we encourage routine sampling of individuals on an annual basis to develop site-specific signatures. Such a "library" of annual signatures would no doubt be useful when identifying the natal origin of long-lived individuals. Finally, while we are encouraged by the use of otolith [Sr] to discriminate between river-spawning walleye stocks in Lake Erie, we strongly recommend continued efforts to expand this approach, as well as the development of others. Quantifying elements besides Sr (e.g., barium, manganese, rubidium) might aid in discrimination between the Maumee and Sandusky Rivers during years in which differences in [Sr] are low (e.g., 2011). Additionally, the use of other natural tagging approaches, such as stable isotope ratios of otoliths (Edmonds and Fletcher 1997, Kennedy et al. 2002) or genetics (Stepien

and Faber 1998, Cadrin and Silva 2005, Bradbury et al. 2008), should be explored to provide multiple possible markers for stock discrimination. With an improved ability to discriminate stocks that could derive such studies, management agencies will be better positioned to determine the origins of fish supporting their population and fisheries. In turn, this information could help agencies better allocate harvest quotas, identify stocks that need to be protected, and determine stocks that need to be rehabilitated, all of which would go a long way towards helping maintain populations at self-sustainable levels.

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Table 1: Sample sizes and means (± 1 standard deviation, SD) for water strontium (parts per billion, ppb) to calcium (parts per million, ppm) ratios (Sr:Ca) and otolith strontium concentration [Sr] in the Maumee and Sandusky Rivers, 1993-2012. Also indicated are those who provided assistance in collecting, processing and/or analyzing samples/data.

Year	Site	Number of Water Samples	Sr:Ca ± SD (ppb:ppm)	Number of Larval Otoliths	Otolith [Sr] ± SD (ppm)
1993	Maumee	1[a]	2.92	20[e]	947 ± 191
	Sandusky	1[a]	4.68	20[e]	1531 ± 151
1994	Maumee	1[a]	3.90	21[e]	701 ± 118
	Sandusky	1[a]	9.26	20[e]	1847 ± 568
1995	Maumee	1[a]	3.47	20[e]	658 ± 177
	Sandusky	-	-	20[e]	1761 ± 223
2001	Maumee	6[b]	10.69 ± 0.27	8[b]	966 ± 218
	Sandusky	4[b]	22.73 ± 0.14	25[b]	2138 ± 862
2011	Maumee	6[c]	8.27 ± 0.91	17[c]	644 ± 163
	Sandusky	6[d]	16.73 ± 2.19	7[d]	953 ± 157
2012	Maumee	8[c]	9.58 ± 2.38	19[c]	946 ± 217
	Sandusky	4	27.07 ± 6.03	19	2173 ± 243

[a] Samples collected and analyzed by USGS National Stream Water-Quality Monitoring Network (Alexander et al. 1996).
[b] Samples collected, processed, and analyzed by K. Hedges and B. Fryer (University of Windsor), and SAL (unpublished data).
[c] Samples collected by J. Pritt, M. DuFour, and C. Mayer (University of Toledo).
[d] Samples collected by J. Davis and J. Miner (Bowling Green State University.
[e] Samples collected by J. Mion and EAM (Ohio State University)

Figure 1: Strontium to calcium ratios (Sr:Ca) in water samples collected from the Maumee and Sandusky Rivers, 1993-2012. Error bars denote ± 1 standard deviation (2001-2012); no replication existed in 1993-1995 collections.

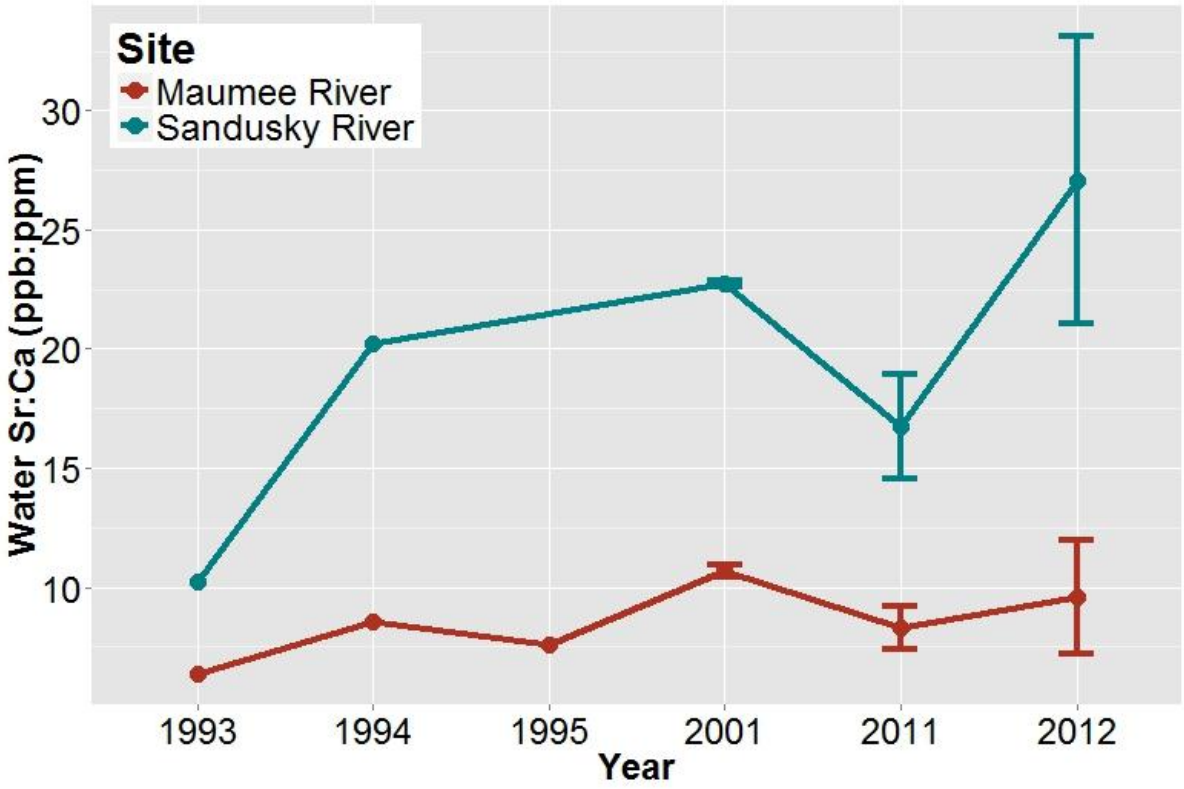

Figure 2: Otolith strontium concentrations [Sr] in larval walleye collected in the Maumee and Sandusky Rivers, 1993-2012. Error bars denote ± 1 standard deviation.

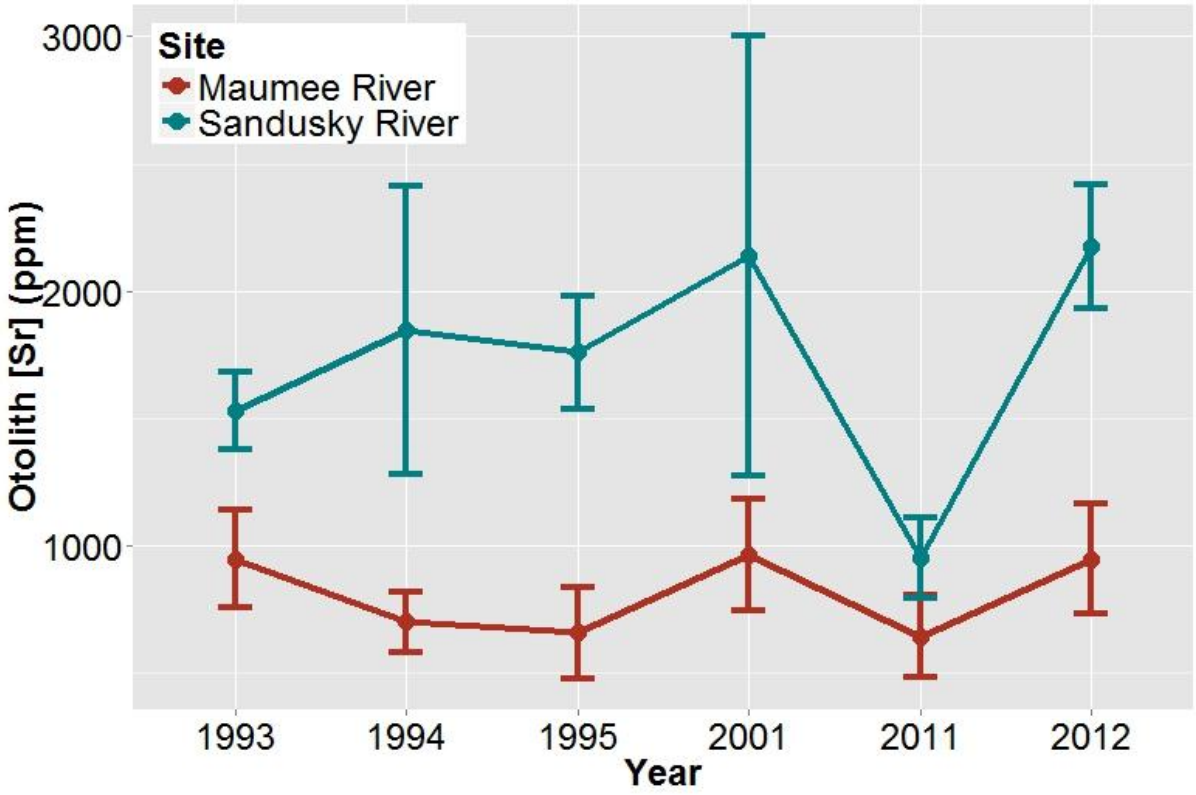

Figure 3: Age versus otolith strontium concentration [Sr] in walleye larvae from the Maumee and Sandusky Rivers, 1993-1995 (top panel) versus 2011-2012 (bottom panel). In the top panel, circles denote larvae sampled during 1993, squares denote larvae sampled during 1994, and triangles denote larvae sampled during 1995. In the bottom panel, circles denote larvae sampled during 2011, whereas square denote larvae sampled during 2012.